

LXXXIII. THE REDUCING PROPERTIES OF ANTISCORBUTIC PREPARATIONS.

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(Received April 29th, 1924.)

In the preceding communication [Zilva, 1924] mention was made of the property of antiscorbutic fractions to reduce ammoniacal silver nitrate in the cold and to decolorise potassium permanganate. The following experiments show that in spite of the close association of the two properties this reducing substance, or substances, is inactivated at a different rate from the antiscorbutic factor. One may therefore conclude that part at least if not all of this reducing material is chemically independent of the physiological principle.

The destruction of the reducing properties of decitrated lemon juice was studied by boiling the juice in the presence and in the absence of air and by exposing it to the action of the atmosphere at ordinary temperature in alkaline medium.

In the first experiment the juice was adjusted to p_H 6.6–6.8 and heated in a boiling water-bath under a reflux condenser. In the aerobic experiments air was aspirated through the boiling solution. In the anaerobic experiments a current of carbon dioxide gas was passed during the heating.

In the second experiment the juice was made $N/20$ alkaline and treated in the way already described [Zilva, 1923].

For the reduction of the potassium permanganate 20 cc. of a 0.5 % solution of the reagent and 10 cc. of 4 N sulphuric acid were added to 0.5 cc. of decitrated lemon juice. The reduction proceeded slowly but it was found that a period of 24 hours was sufficient to complete it. The flasks were kept in the laboratory at ordinary temperature and additional permanganate was added if necessary. The residual potassium permanganate was titrated with sodium thiosulphate.

In the silver reduction experiments 5 cc. of $N/14$ ammoniacal silver nitrate solution were added to the decitrated lemon juice and allowed to remain for 20 minutes (reduction is complete after 15 minutes). A crystal of barium nitrate and 4 cc. of $N/10$ nitric acid were added to agglutinate the reduced

silver (p_H 7.2-7.4). After filtering off the precipitate a further 5 cc. of $N/10$ nitric acid was added and the unreduced silver nitrate was titrated with $N/10$ sodium chloride, using potassium chromate as an outside indicator. It was found that the square of the amount of silver reduced (expressed in cc. of the solution) was proportional to the volume of the decitrated lemon juice used, and the constant obtained by the ratio of the former to the latter was used as a measure of the amount of reduction. This is illustrated by the following figures:

	Volume of decitrated juice taken cc.	Amount of reduction in cc. $N/10$ $AgNO_3$	(Amount of reduction) ² Vol. of solution taken
I.	1	.3	.09
	2.5	.5	.10
	5	.7	.10
	10	1.2	.14
	20	1.6	.12
II.	2.5	.8	.25
	5	1.2	.29
	10	1.7	.29
	20	2.0	.20

Tables I and II give the details of the experiments.

Table I. *Inactivation by heat.*

Solution	Percentage of silver-reducing substance destroyed	Percentage of per- manganate-reducing substance destroyed
Original	0	0
Heated in CO_2 for 3 hours	0	0
Heated in air for 1 hour	25 ± 15	5
Heated in air for 3 hours	40 ± 20	5

Table II. *Inactivation by alkalinity.*

Solution	Percentage of silver-reducing substance destroyed	Percentage of per- manganate-reducing substance destroyed
Original	0	0
Standing 24 hours in vacuum	0	0
Standing 1 hour in air	30 ± 12	6
Standing 3 hours in air	45 ± 25	6

It will be seen that the material responsible for the decolorisation of the potassium permanganate is very little affected by either treatment. A bigger impression was made on the silver reducing substance, although a marked variation is recorded in this respect in the various samples. Even the highest figure, however, is considerably lower than that obtained in the inactivation of the antiscorbutic factor under similar conditions [Zilva, 1922, 1923].

In this connection reference may be made to the observation recorded by Bezssonoff [1922] that certain antiscorbutic substances produce a blue coloration with a modified Folin's reagent for phenol, which is evidently a reduction process. He suggested that this reaction might be utilised as a test for the

antiscorbutic factor. Kay and Zilva [1923] have since shown that certain antiscorbutic preparations failed to give this reaction whilst substances such as yeast which, as is well known, do not possess antiscorbutic properties, produce the coloration. Bezssonoff [1924] in a subsequent communication modifies his view and asserts that the coloration is produced by a degradation product of the antiscorbutic factor and that if an active substance fails to give the reaction directly it will do so after heating. He also criticised Kay and Zilva's observation on the behaviour of yeast towards the reagent, asserting that his improved reagent [1923] does not give a coloration with yeast.

We have repeated the experiments with the yeast, using the improved reagent, and, as before, we obtained a coloration. Heating the adsorbed antiscorbutic preparation used by Kay and Zilva on the other hand again failed to give the colour. Further active fractions as obtained in Exps. 3, 6 and 7 [Zilva, 1924] also gave negative results before and after heating. The reaction is therefore useless for the purpose of establishing the presence of the antiscorbutic factor.

Thanks are due to the Medical Research Council for a whole time grant.

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